In the claims:

1. (Currently amended) A fluorescein-based ligand, comprising a ligand having <u>one</u> of the following structures:

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$$ZO$$
 K
 QZ
 II

<u>or</u>

wherein, independently for each occurrence:

A is a chemical moiety having one or more to about ten carbon atoms;

Z is hydrogen or any hydroxyl-protecting group;

Q is O, S or Se;

K is optionally one or more of the following substituents of the indicated aromatic ring: aliphatic, alkyl, aralkyl, alkenyl, alkynyl, aryl, amine, acyl, acyloxy, acylamino, amido, alkylthio, carbonyl, alkoxyl, sulfonate, sulfate, sulfamoyl, sulfonyl, sulfoxido, selenoalkyl, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl, sulfonyl and trifluoromethylthat do not preclude detecting a metal ion by fluorescence;

V is a chemical moiety comprising a Lewis base capable of forming one or more coordination bonds with a metal ion (i) at least three Lewis bases that are capable of forming a tridentate chelating agent with one metal ion, wherein at least two of said three Lewis bases form a coordination bond with said metal ion through a nitrogen atom and at least one of said nitrogen atoms is in the ring structure of an aryl heterocycle, or (ii) a secondary nitrogen atom doubly bonded to a carbon atom of A to form an imine, wherein said secondary nitrogen atom is capable of forming a bidentate chelating agent with the oxygen atom of OZ or Q;

Y is O, S, Se, NR, or $[C(CH_3)_3]$ $\underline{C(CH_3)_2}$, wherein R is an alkyl and R and the methyl groups of $C(CH_3)_2$ are optionally substituted; and

Z2 is N, HOOCCH₂CH₂C-, HOOC-CH=CH-C-, (2-carboxyphenyl)-C-, or (2-sulfophenyl)-C-, (2-carboxy-3,4,5,6-tetrachlorophenyl)-C-, (2-carboxy-4-nitrophenyl)-C-, (2-carboxy-5-nitrophenyl)-C-, (2-carboxy-4-aminophenyl)-C-, (2-carboxy-5-aminophenyl)-C-, (2,4-dicarboxyphenyl)-C-, (2,5-dicarboxylphenyl)-C-, (2,4,5-tricarboxyphenyl)-C-, or other substituted (2-carboxyphenyl)-C- derivative wherein for said (2-carboxyphenyl)-C- and (2-sulfophenyl)-C-, said phenyl moiety is optionally substituted with one or more E, and wherein for said HOOCCH₂CH₂C- and HOOC-CH=CH-C-, said hydrogen atoms of said -CH₂-'s and -CH='s are optionally substituted and said HOOC-'s are optionally a carbonyl other than carboxylic acid;

E is optionally one or more of the following substituents of the indicated aromatic ring: aliphatic, alkyl, aralkyl, alkenyl, alkynyl, aryl, amine, acyl, acyloxy, acylamino, amido, alkylthio, carbonyl, alkoxyl, sulfonate, sulfate, sulfamoyl, sulfonyl, sulfoxido, selenoalkyl, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl, sulfonyl and trifluoromethyl; and

D is -CH₂CH₂- or -CH=CH-, wherein said hydrogen atoms are optionally substituted.

- (Currently amended) The fluorescein-based ligand of claim 1, wherein A is one of the following, optionally substituted: -CH2-, -C(=O)-, -C(=S)-, -CH2CH2-, -CH2C(=O)-, -CH2C(=S)-, and -C(H)=, wherein Y is O, and wherein Q is O and optionally J is substituted for any hydrogen atom of said -CH2-, -CH2CH2-, -CH2C(=O)-, or -CH2C(=S)-, wherein J is a non-interfering substituent.
- 3. (Currently amended) The fluorescein-based ligand of claim [1] 2, wherein: said ligand has one or more K substituents, with each K independently being one of the following: linear or branched alkyl, alkenyl, linear or branched aminoalkyl, linear or branched acylamino, linear or branched acyloxy, linear or branched alkoxycarbonyl, linear or branched alkoxy, linear or branched alkylaryl, linear or branched hyrdoxyalkyl, linear or branched thioalkyl, acyl, amino, hydroxy, thio, aryloxy, arylalkoxy, hydrogen, alkynyl, halogen, cyano, sulfhydryl, carbamoyl, nitro, trifluoromethyl, amino, thio, lower alkoxy, lower alkylthio, lower alkylamino, nitro, phenoxy, benzyloxy, hydrogen, amine, hydroxyl, alkoxyl, carbonyl, acyl, formyl or sulfonyl said ligand has formula I or II; K is optionally one or more of the following substituents of the indicated aromatic ring: halogen; Z2 is (2-carboxyphenyl)-C-; and E is optionally one or more of the following substituents of the indicated aromatic ring: amido, carbonyl and halogen.
- 4. (Currently amended) The fluorescein-based ligand of claim [1] 3, wherein Q is OZ and Z2 forms a different tautomer with the fluorescein core K is present as halogen in either or both of the 2' and 7' positions of said ligand, and wherein E is not present.
- 5. (Currently amended) The fluorescein based-ligand of claim 1, wherein: Q is O; Y is O; and V is a chemical moiety comprising at least three Lewis bases that are capable of forming a tridentate chelating agent with one metal ion, wherein at least two of said three Lewis bases form a coordination bond with said metal ion through a nitrogen atom and both of said nitrogen atoms are in the ring structure of the same or different aryl heterocycles. said ligand has the following structure:

- 6. (Currently amended) The fluorescein-based ligand of claim [5] 1, wherein: Y is O; Q is O; Z is H; and V is a chemical moiety comprising at least three Lewis bases that are capable of forming a tridentate chelating agent with one metal ion, wherein at least two of said three Lewis bases form a coordination bond with said metal ion through a nitrogen atom, at least one of said nitrogen atoms is in the ring structure of an aryl heterocycle, and said tridentate chelating agent is capable of forming a tetradentate chelating agent with the oxygen atom of OZ or Q. A is one of the following: CH2-, C(=O) , C(=S) , CH2CH2-, CH2C(=O) , CH2C(=S) , and optionally J is substituted for any -H of CH2-, CH2CH2-, CH2C(=O) , CH2C(=S) , wherein J is a non-interfering substituent.
- 7. (Currently amended) The fluorescein-based ligand of claim [5] 3, wherein K is present at both the 2' and 7' positions of said ligand said ligand has one or more K substituents, with each K independently being either -Cl or -F.
- 8. (Currently amended) The fluorescein-based ligand of claim 5, wherein: said ligand has formula I or II; K is optionally one or more of the following substituents of the indicated aromatic ring: halogen; Z2 is (2-carboxyphenyl)-C-; and E is optionally one or more of the following substituents of the indicated aromatic ring: amido, carbonyl and halogen the fluorescein structure of said ligand is in a different isomeric form.
- 9. (Currently amended) The fluorescein-based ligand of claim [1] 8, wherein K is present in one or both of the indicated aromatic rings as halogen said ligand forms a chelating agent upon complexation of said ligand with a metal ion.

- 10. (Currently amended) The fluorescein-based ligand of claim [9] 8, wherein E is present in said ligand as either one carbonyl or one amido said ligand forms a tridentate or tetradentate chelating agent upon said complexation.
- 11. (Currently amended) The fluorescein-based ligand of claim 1, wherein said ligand has one of the following structures:

wherein K is optionally one or more of the following substituents of the indicated aromatic ring: halogen R1 is aliphatic, alkyl, aralkyl, alkenyl, alkynyl, aryl or heterocyclyl, optionally substituted.

12. (Withdrawn) A fluorescein-based compound for a target, comprising the following structure:

wherein: A is a moiety having one or more carbon atoms;

Z is hydrogen or any hydroxyl-protecting group;

Q is O, S or Se;

K is optionally one or more substituents of the indicated aromatic ring that that do not preclude using said compound for detecting a target;

V is a chemical moiety that may serve as a ligand to said target;

Y is O, S, Se, NR, or C(CH3)3, wherein R is an alkyl and R and the methyl groups of C(CH3)2 are optionally substituted; and

Z2 is N, HOOCCH₂CH₂C-, HOOC-CH=CH-C-, (2-carboxyphenyl)-C-, (2-sulfophenyl)-C-, (2-carboxy-3,4,5,6-tetrachlorophenyl)-C-, (2-carboxy-4-nitrophenyl)-C-, (2-carboxy-5-nitrophenyl)-C-, (2-carboxy-4-aminophenyl)-C-, (2-carboxy-5-aminophenyl)-C-, (2,4-dicarboxyphenyl)-C-, (2,5-dicarboxylphenyl)-C-, (2,4,5-tricarboxyphenyl)-C-, or other substituted (2-carboxyphenyl)-C- derivative.

- 13. (Withdrawn) The fluorescein-based compound of claim 12, wherein said target is a biological material.
- 14. (Withdrawn) The fluorescein-based compound of claim 12, wherein said target is a toxin.

15. (Withdrawn) A fluorescein-based scaffold, comprising a scaffold having the following structure:

wherein: Z is hydrogen or any hydroxyl-protecting group;

Q is O, S or Se;

K is optionally one or more substituents of the indicated aromatic ring that do not preclude detecting a metal ion by fluorescence;

W comprises at least one carbon atom bound to the aromatic ring carbon and is a site of latent functionality;

Y is O, S, Se, NR, or C(CH3)3, wherein R is an alkyl and R and the methyl groups of C(CH3)2 are optionally substituted; and

Z2 is N, HOOCCH₂CH₂C-, HOOC-CH=CH-C-, (2-carboxyphenyl)-C-, (2-sulfophenyl)-C-, (2-carboxy-3,4,5,6-tetrachlorophenyl)-C-, (2-carboxy-4-nitrophenyl)-C-, (2-carboxy-5-nitrophenyl)-C-, (2-carboxy-4-aminophenyl)-C-, (2-carboxy-5-aminophenyl)-C-, (2,4-dicarboxyphenyl)-C-, (2,5-dicarboxylphenyl)-C-, (2,4,5-tricarboxyphenyl)-C-, or other substituted (2-carboxyphenyl)-C- derivative.

16. (Withdrawn) The fluorescein-based scaffold of claim 15, wherein said scaffold has the following structure:

17. (Withdrawn) The fluorescein-based scaffold of claim 16, wherein W comprises one of the following: -CH2X, -C(O)H, -C(O)OR2, -C(O)OH, -C(O)X, -CN

- wherein X is halogen, hydroxyl, amine, thiol or other leaving group, and R2 is an aliphatic, alkyl, aralkyl, alkenyl, alkynyls, aryl or heterocyclyl.
- 18. (Withdrawn) The fluorescein-based scaffold of claim 16, wherein the fluorescein structure of said scaffold is in a different isomeric form.
- 19. (Withdrawn) The fluorescein-based scaffold of claim 15, wherein said scaffold has one of the following structures:

wherein X is halogen, hydroxyl, amine, thiol or other leaving group.

20. (Currently amended) A fluorescein-based ligand, comprising a ligand having <u>one</u> of the following structures:

wherein, independently for each occurrence:

A is a chemical moiety having one or more to about ten carbon atoms;

Z is hydrogen or any hydroxyl-protecting group;

Q is O, S or Se;

K is optionally one or more of the following substituents of the indicated aromatic ring: aliphatic, alkyl, aralkyl, alkenyl, alkynyl, aryl, amine, acyl, acyloxy, acylamino, amido, alkylthio, carbonyl, alkoxyl, sulfonate, sulfate, sulfamoyl, sulfonyl, sulfoxido, selenoalkyl, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl, sulfonyl and trifluoromethylthat do not preclude detecting a metal ion by fluorescence;

V is a chemical moiety comprising a Lewis base capable of forming one or more coordination bonds with a metal ion (i) at least three Lewis bases that are capable of forming a tridentate chelating agent with one metal ion, wherein at least two of said three Lewis bases form a coordination bond with said metal ion through a nitrogen atom and at least one of said nitrogen atoms is in the ring structure of an aryl heterocycle, or (ii) a secondary nitrogen atom doubly

bonded to a carbon atom of A to form an imine, wherein said secondary nitrogen atom is capable of forming a bidentate chelating agent with OZ;

Y is O, S, Se, NR, or [C(CH3)3] <u>C(CH3)2</u>, wherein R is an alkyl and R and the methyl groups of C(CH3)2 are optionally substituted; and

Z2 is N, HOOCCH₂CH₂C-, HOOC-CH=CH-C-, (2-carboxyphenyl)-C-, or (2-sulfophenyl)-C-, (2-carboxy-3,4,5,6-tetrachlorophenyl)-C-, (2-carboxy-4-nitrophenyl)-C-, (2-carboxy-5-nitrophenyl)-C-, (2-carboxy-5-aminophenyl)-C-, (2-carboxyphenyl)-C-, or other substituted (2-carboxyphenyl)-C- derivative wherein for said (2-carboxyphenyl)-C- and (2-sulfophenyl)-C-, said phenyl moiety is optionally substituted with one or more E, and wherein for said HOOCCH₂CH₂C- and HOOC-CH=CH-C-, said hydrogen atoms of said -CH₂-'s and -CH='s are optionally substituted and said HOOC-'s are optionally a carbonyl other than carboxylic acid;

E is optionally one or more of the following substituents of the indicated aromatic ring: aliphatic, alkyl, aralkyl, alkenyl, alkynyl, aryl, amine, acyl, acyloxy, acylamino, amido, alkylthio, carbonyl, alkoxyl, sulfonate, sulfate, sulfamoyl, sulfonyl, sulfoxido, selenoalkyl, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl, sulfonyl and trifluoromethyl; and

D is -CH₂CH₂- or -CH=CH-, wherein said hydrogen atoms are optionally substituted.

- 21. (Currently amended) The fluorescein-based ligand of claim 20, wherein A is one of the following, optionally substituted: -CH2-, -C(=O)-, -C(=S)-, -CH2CH2-, -CH2C(=O)-, -CH2C(=S)-, and -C(H)=, wherein Y is O, and wherein Q is O and optionally J is substituted for any hydrogen atom of said -CH2-, -CH2CH2-, -CH2C(=O)-, or -CH2C(=S)-, wherein J is a non-interfering substituent.
- 22. (Currently amended) The fluorescein-based ligand of claim [20] 21, wherein: said ligand has one or more K substituents, with each K independently being one of the following: linear or branched alkyl, alkenyl, linear or branched aminoalkyl, linear or branched acylamino, linear or branched acyloxy, linear or branched alkoxycarbonyl, linear or branched alkoxy, linear or branched alkylaryl, linear or

branched hyrdoxyalkyl, linear or branched thioalkyl, acyl, amino, hydroxy, thio, aryloxy, arylalkoxy, hydrogen, alkynyl, halogen, cyano, sulfhydryl, carbamoyl, nitro, trifluoromethyl, amino, thio, lower alkoxy, lower alkylthio, lower alkylamino, nitro, phenoxy, benzyloxy, hydrogen, amine, hydroxyl, alkoxyl, earbonyl, acyl, formyl or sulfonyl said ligand has formula I or II; K is optionally one or more of the following substituents of the indicated aromatic ring: halogen; Z2 is (2-carboxyphenyl)-C-; and E is optionally one or more of the following substituents of the indicated aromatic ring: amido, carbonyl and halogen.

- 23. (Currently amended) The fluorescein-based ligand of claim [20] 22, wherein Q is -OZ and Z2 forms a different tautomer with the fluorescein core K is present as halogen in either or both of the 2' and 7' positions of said ligand, and wherein E is not present.
- 24. (Currently amended) The fluorescein based-ligand of claim 20, wherein: Q is O; Y is O; and V is a chemical moiety comprising at least three Lewis bases that are capable of forming a tridentate chelating agent with one metal ion, wherein at least two of said three Lewis bases form a coordination bond with said metal ion through a nitrogen atom and both of said nitrogen atoms are in the ring structure of the same or different aryl heterocycles. said ligand has the following structure:

25. (Currently amended) The fluorescein-based ligand of claim [24] 20, wherein: Y is O; Q is O; Z is H; and V is a chemical moiety comprising at least three Lewis bases that are capable of forming a tridentate chelating agent with one metal ion, wherein at least two of said three Lewis bases form a coordination bond with said metal ion through a nitrogen atom, at least one of said nitrogen atoms is in the

- ring structure of an aryl heterocycle, and said tridentate chelating agent is capable of forming a tetradentate chelating agent with OZ. A is one of the following:

 CH2-, C(=O), C(=S), CH2CH2-, CH2C(=O), CH2C(=S), and optionally J is substituted for any H of CH2-, CH2CH2-, CH2C(=O), CH2C(=S), wherein J is a non-interfering substituent.
- 26. (Currently amended) The fluorescein-based ligand of claim [24] <u>22</u>, wherein <u>K is present at both the 2' and 7' positions of said ligand said ligand has one or more K substituents, with each K independently being either -Cl or F.</u>
- 27. (Currently amended) The fluorescein-based ligand of claim 24, wherein: said ligand has formula I or II; K is optionally one or more of the following substituents of the indicated aromatic ring: halogen; Z2 is (2-carboxyphenyl)-C-; and E is optionally one or more of the following substituents of the indicated aromatic ring: amido, carbonyl and halogen the fluorescein structure of said ligand is in a different isomeric form.
- 28. (Currently amended) The fluorescein-based ligand of claim [20] <u>27</u>, wherein <u>K is present in one or both of the indicated aromatic rings as halogen said ligand forms a chelating agent upon complexation of said ligand with a metal ion.</u>
- 29. (Currently amended) The fluorescein-based ligand of claim [28] <u>27</u>, wherein <u>E is present in said ligand as either one carbonyl or one amido said ligand forms a tridentate or tetradentate chelating agent upon said complexation.</u>
- 30. (Currently amended) The fluorescein-based ligand of claim 20, wherein said ligand has one of the following structures:

wherein K is optionally one or more of the following substituents of the indicated aromatic ring: halogen R1 is aliphatic, alkyl, aralkyl, alkenyl, alkynyl, aryl or heterocyclyl, optionally substituted.

31. (Withdrawn) A fluorescein-based compound for a target, comprising the following structure:

wherein: A is a moiety having one or more carbon atoms;

Z is hydrogen or any hydroxyl-protecting group;

Q is O, S or Se;

K is optionally one or more substituents of the indicated aromatic ring that that do not preclude using said compound for detecting a target;

V is a chemical moiety that may serve as a ligand to said target;

Y is O, S, Se, NR, or C(CH3)3, wherein R is an alkyl and R and the methyl groups of C(CH3)2 are optionally substituted; and

Z2 is N, HOOCCH₂CH₂C-, HOOC-CH=CH-C-, (2-carboxyphenyl)-C-, (2-sulfophenyl)-C-, (2-carboxy-3,4,5,6-tetrachlorophenyl)-C-, (2-carboxy-4-nitrophenyl)-C-, (2-carboxy-5-nitrophenyl)-C-, (2-carboxy-4-aminophenyl)-C-, (2-carboxy-5-aminophenyl)-C-, (2,4-dicarboxyphenyl)-C-, (2,5-dicarboxylphenyl)-C-, (2,4,5-tricarboxyphenyl)-C-, or other substituted (2-carboxyphenyl)-C- derivative.

- 32. (Withdrawn) The fluorescein-based compound of claim 31, wherein said target is a biological material.
- 33. (Withdrawn) The fluorescein-based compound of claim 31, wherein said target is a toxin.
- 34. (Withdrawn) A fluorescein-based scaffold, comprising a scaffold having the following structure:

wherein: Z is hydrogen or any hydroxyl-protecting group;

Q is O, S or Se;

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K is optionally one or more substituents of the indicated aromatic ring that do not preclude detecting a metal ion by fluorescence;

W comprises at least one carbon atom bound to the aromatic ring carbon and is a site of latent functionality;

Y is O, S, Se, NR, or C(CH3)3, wherein R is an alkyl and R and the methyl groups of C(CH3)2 are optionally substituted; and

Z2 is N, HOOCCH₂CH₂C-, HOOC-CH=CH-C-, (2-carboxyphenyl)-C-, (2-sulfophenyl)-C-, (2-carboxy-3,4,5,6-tetrachlorophenyl)-C-, (2-carboxy-4-nitrophenyl)-C-, (2-carboxy-5-nitrophenyl)-C-, (2-carboxy-4-aminophenyl)-C-, (2-carboxy-5-aminophenyl)-C-, (2,4-dicarboxyphenyl)-C-, (2,5-dicarboxylphenyl)-C-, (2,4,5-tricarboxyphenyl)-C-, or other substituted (2-carboxyphenyl)-C- derivative.

35. (Withdrawn) The fluorescein-based scaffold of claim 34, wherein said scaffold has the following structure:

- 36. (Withdrawn) The fluorescein-based scaffold of claim 34, wherein W comprises one of the following: -CH2X, -C(O)H, -C(O)OR2, -C(O)OH, -C(O)X, -CN wherein X is halogen, hydroxyl, amine, thiol or other leaving group, and R2 is an aliphatic, alkyl, aralkyl, alkenyl, alkynyls, aryl or heterocyclyl.
- 37. (Withdrawn) The fluorescein-based scaffold of claim 35, wherein the fluorescein structure of said scaffold is in a different isomeric form.
- 38. (Withdrawn) The fluorescein-based scaffold of claim 35, wherein said scaffold has one of the following structures:

wherein X is halogen, hydroxyl, amine, thiol or other leaving group.

- 39. (Withdrawn) A coordination complex, comprising a metal ion coordinated to one of the fluorescein-based ligands claimed above.
- 40. (Withdrawn) A coordination complex, comprising Zn2+ coordinated to one of the fluorescein-based ligands claimed above.
- 41. (Withdrawn) A method of detecting, and optionally quantifying the concentration of, a metal ion in a sample, comprising:
- a. Adding to a sample a fluorescein-based ligand comprising one of the following structures:

$$zo$$
 X
 Y
 Zz
 X
 Zz
 X
 Zz

wherein: A is a moiety having one or more carbon atoms;

Z is hydrogen or any hydroxyl-protecting group;

Q is O, S or Se;

K is optionally one or more substituents of the indicated aromatic ring that do not preclude detecting a metal ion by fluorescence;

V is a chemical moiety comprising a Lewis base capable of forming one or more coordination bonds with a metal ion;

Y is O, S, Se, NR, or C(CH3)3, wherein R is an alkyl and R and the methyl groups of C(CH3)2 are optionally substituted; and

Z2 is N, HOOCCH₂CH₂C-, HOOC-CH=CH-C-, (2-carboxyphenyl)-C-, (2-sulfophenyl)-C-, (2-carboxy-3,4,5,6-tetrachlorophenyl)-C-, (2-carboxy-4-nitrophenyl)-C-, (2-carboxy-5-nitrophenyl)-C-, (2-carboxy-4-aminophenyl)-C-, (2-carboxy-5-aminophenyl)-C-, (2,4-dicarboxyphenyl)-C-, (2,5-dicarboxylphenyl)-C-, (2,4,5-tricarboxyphenyl)-C-, or other substituted (2-carboxyphenyl)-C- derivative; and

- b. Measuring the fluorescence of said ligand in said sample; and
- c. Determining whether a metal ion is present in said sample, and optionally the concentration of said metal ion in said sample.
- 42. (Withdrawn) The method of claim 41, wherein said sample is a cell.
- 43. (Withdrawn) The method of claim 41, wherein said sample is <u>in vitro</u>.
- 44. (Withdrawn) The method of claim 41, further comprising measuring the fluorescence of said ligand in said sample at a different concentration of said ligand.
- 45. (Withdrawn) The method of claim 41, wherein one of said metal ions is a transition metal ion.
- 46. (Withdrawn) The method of claim 41, wherein one of said metal ions is Zn2+.
- 47. (Withdrawn) A method of detecting, and optionally quantifying the concentration of, a target in a sample, comprising:
- a. Mixing with a sample a fluorescein-based compound comprising one of the following structures:

wherein: A is a moiety having one or more carbon atoms;

Z is hydrogen or any hydroxyl-protecting group;

Q is O, S or Se;

K is optionally one or more substituents of the indicated aromatic ring that that do not preclude using said compound for detecting a target;

V is a chemical moiety that may serve as a ligand to said target;

Y is O, S, Se, NR, or $C(CH_3)_3$, wherein R is an alkyl and R and the methyl groups of $C(CH_3)_2$ are optionally substituted; and

Z2 is N, HOOCCH₂CH₂C, HOOC-CH=CH-C-, (2-carboxyphenyl)-C-, (2-sulfophenyl)-C-, (2-carboxy-3,4,5,6-tetrachlorophenyl)-C-, (2-carboxy-4-nitrophenyl)-C-, (2-carboxy-5-nitrophenyl)-C-, (2-carboxy-4-aminophenyl)-C-, (2-carboxy-5-aminophenyl)-C-, (2,4-dicarboxyphenyl)-C-, (2,5-dicarboxylphenyl)-C-, (2,4,5-tricarboxyphenyl)-C-, or other substituted (2-carboxyphenyl)-C- derivative; and

- b. Measuring the fluorescence of said compound in said sample; and
- c. Determining whether the target is present in said sample, and optionally the concentration of said target in said sample.
- 48. (Currently amended) A diagnostic kit for a metal ion, comprising:
- a. A fluorescein-based ligand comprising one of the following structures:

$$z_0$$
 y
 z_2
 y
 z_2
 y
 z_2

I

$$\frac{z_0}{\frac{V}{E}}$$

wherein, independently for each occurrence:

A is a chemical moiety having one or more to about ten carbon atoms;

Z is hydrogen or any hydroxyl-protecting group;

Q is O, S or Se;

K is optionally one or more of the following substituents of the indicated aromatic ring: aliphatic, alkyl, aralkyl, alkenyl, alkynyl, aryl, amine, acyl, acyloxy, acylamino, amido, alkylthio, carbonyl, alkoxyl, sulfonate, sulfate, sulfamoyl, sulfonyl, sulfoxido, selenoalkyl, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl, sulfonyl and trifluoromethylthat do not preclude detecting a metal ion by fluorescence;

V is a chemical moiety comprising a Lewis base capable of forming one or more coordination bonds with a metal ion (i) at least three Lewis bases that are capable of forming a tridentate chelating agent with one metal ion, wherein at least two of said three Lewis bases form a coordination bond with said metal ion through a nitrogen atom and at least one of said nitrogen

atoms is in the ring structure of an aryl heterocycle, or (ii) a secondary nitrogen atom doubly bonded to a carbon atom of A to form an imine, wherein said secondary nitrogen atom is capable of forming a bidentate chelating agent with the oxygen atom of OZ or Q;

Y is O, S, Se, NR, or [C(CH3)3] <u>C(CH3)2</u>, wherein R is an alkyl and R and the methyl groups of C(CH3)2 are optionally substituted; and

Z2 is N, HOOCCH₂CH₂C-, HOOC-CH=CH-C-, (2-carboxyphenyl)-C-, or (2-sulfophenyl)-C-, (2-carboxy-3,4,5,6-tetrachlorophenyl)-C-, (2-carboxy-4-nitrophenyl)-C-, (2-carboxy-5-nitrophenyl)-C-, (2-carboxy-4-aminophenyl)-C-, (2-carboxy-5-aminophenyl)-C-, or other substituted (2-carboxyphenyl)-C-, or other substituted (2-carboxyphenyl)-C- derivative wherein for said (2-carboxyphenyl)-C- and (2-sulfophenyl)-C-, said phenyl moiety is optionally substituted with one or more E, and wherein for said HOOCCH₂CH₂C- and HOOC-CH=CH-C-, said hydrogen atoms of said -CH₂-'s and -CH='s are optionally substituted and said HOOC-'s are optionally a carbonyl other than carboxylic acid;

E is optionally one or more of the following substituents of the indicated aromatic ring: aliphatic, alkyl, aralkyl, alkenyl, alkynyl, aryl, amine, acyl, acyloxy, acylamino, amido, alkylthio, carbonyl, alkoxyl, sulfonate, sulfate, sulfamoyl, sulfonyl, sulfoxido, selenoalkyl, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl, sulfonyl and trifluoromethyl; and

D is -CH₂CH₂- or -CH=CH-, wherein said hydrogen atoms are optionally substituted; and

- b. Instructions for using said ligand to detect a metal ion in a sample.
- 49.. (Withdrawn) A diagnostic kit for a target, comprising:
- a. A fluorescein-based compound comprising one of the following structures:

wherein: A is a moiety having one or more carbon atoms;

Z is hydrogen or any hydroxyl-protecting group;

Q is O, S or Se;

K is optionally one or more substituents of the indicated aromatic ring that that do not preclude using said compound for detecting a target;

V is a chemical moiety that may serve as a ligand to said target;

Y is O, S, Se, NR, or C(CH3)3, wherein R is an alkyl and R and the methyl groups of C(CH3)2 are optionally substituted; and

Z2 is N, HOOCCH₂CH₂C, HOOC-CH=CH-C, (2-carboxyphenyl)-C, (2-sulfophenyl)-C, (2-carboxy-3,4,5,6-tetrachlorophenyl)-C, (2-carboxy-4-nitrophenyl)-C, (2-carboxy-5-nitrophenyl)-C, (2-carboxy-4-aminophenyl)-C, (2-carboxy-5-aminophenyl)-C, (2,4-dicarboxyphenyl)-C, (2,5-dicarboxylphenyl)-C, (2,4,5-tricarboxyphenyl)-C, or other substituted (2-carboxyphenyl)-C derivative; and

b. Instructions for using said compound to detect a target in a sample.